

## Mast cell number and phenotype in chronic idiopathic urticaria

By: Catherine H. Smith, [Christopher Kepley](#), Lawrence B. Schwartz, and Tak H. Lee

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### Abstract:

**Background:** Increased levels of histamine have been previously demonstrated in patients with chronic idiopathic urticaria. **Objective:** The purpose of the study was to determine whether increased numbers of mast cells are present in lesional skin from such patients. **Methods:** Mast cells have been quantified in lesional ( $n = 11$ ) and nonlesional ( $n = 9$ ) skin from patients with chronic idiopathic urticaria and compared with site-matched skin from healthy control subjects ( $n = 10$ ). Mast cells were identified by using a sensitive, double-labeling immunohistochemical technique with specific monoclonal antibodies to mast cell tryptase and chymase and quantified under light microscopy. **Results:** No significant differences in mast cell numbers from lesional, nonlesional, or control skin were observed ( $p > 0.1$ , Student's  $t$  test). In both patients with urticaria and control subjects, more than 99% of cutaneous mast cells contained tryptase and chymase. **Conclusions:** These data indicate that increased skin histamine in chronic idiopathic urticaria is not caused by increased mast cells and may alternatively reflect an increase in histamine content per mast cell, enhanced mast cell activation, or recruitment of basophils into skin in patients with chronic idiopathic urticaria.

**Keywords:** Urticaria | mast cells | MC<sub>TC</sub> | MC<sub>T</sub> | immunohistochemistry

### Article:

Chronic idiopathic urticaria is characterized by recurrent, itchy, evanescent wheals, occurring over a period of more than 6 weeks. Much evidence indicates a central role for cutaneous mast cells, although until recently, triggers of mast cell degranulation remained obscure in the majority of patients. More recent evidence, however, suggests that more than 50% of patients have circulating autoantibodies directed against IgE, the alpha subunit of the high-affinity IgE receptor (FcεRI), or both molecules; these autoantibodies have functional histamine-releasing activity.<sup>1,2</sup> Others report the presence of histamine releasing factor in suction blister fluid overlying lesional and nonlesional skin of patients with active urticaria.<sup>3,4</sup> This activity may be related to the presence of anti-IgE or anti-FcεRI autoantibodies in skin or may alternatively

reflect release of chemokines, such as macrophage inflammatory protein-1 $\alpha$ , which have been shown to release histamine from mast cells.<sup>5,6</sup> Regardless of the exact nature of the factor or factors responsible for mast cell mediator release, histamine remains of crucial pathogenetic importance in urticaria. Most patients respond to specific H<sub>1</sub>-receptor antagonists. Intradermal histamine exactly reproduces the symptoms (pruritus) and signs (wheal and flare) of urticaria.<sup>7</sup> Furthermore, increased levels of histamine have been demonstrated in nonlesional and lesional skin in patients with chronic idiopathic urticaria.<sup>8,9,10</sup> This increase in skin histamine has been postulated to be due to an increase in the number of cutaneous mast cells. However, quantification of mast cells in vivo is complicated by the tendency of the cells to degranulate, such that their characteristic metachromatic staining is diminished or lost. Specific monoclonal antibodies to mast cell tryptase and chymase, suitable for use in immunohistochemical techniques, have recently become available<sup>11</sup> and afford a high degree of specificity and sensitivity for mast cell detection. These antibodies were therefore used to assess mast cell numbers in nonlesional and lesional skin in patients with chronic idiopathic urticaria and in skin from healthy control subjects to investigate further the origin of observed increases in skin histamine in patients with urticaria.

## **METHODS**

### **Subjects**

Eleven patients with chronic idiopathic urticaria were recruited (6 men and 5 women; age range, 21 to 63 years), and nine consented to skin biopsies of both lesional and nonlesional skin. Chronic idiopathic urticaria was defined as the appearance of recurrent, multiple cutaneous wheals for more than 3 months, occurring at intervals of not less than 1 week. All patients were subject to a detailed history, physical examination, and as appropriate, laboratory investigation to exclude underlying causes of urticaria. Exclusion criteria included a history of or clinical evidence for predominantly physical urticarias, urticaria occurring only after ingestion of a specific drug or food, or clear improvement in urticaria with an additive-free and preservative-free diet. To avoid inclusion of patients with urticarial vasculitis, patients with a history of wheals lasting more than 24 hours, systemic symptoms, or signs of purpura or bruising at sites of urticaria were excluded. Antihistamines were discontinued for 48 hours before the day of study, and in the case of astemizole, for 6 weeks before the study day. Ten healthy control subjects were also recruited (3 men and 7 women; age range, 25 to 60 years).

### **Ethical approval**

All studies performed were approved by the Guy's Hospital Ethics Committee and involved subjects who had given informed, written consent.

### **Skin biopsy**

Patients with urticaria underwent skin biopsy of an urticarial wheal within 12 hours of its appearance. Site-matched nonlesional skin (not subject to recent whealing) and healthy skin were also biopsied in patients and healthy control subjects, respectively. With the exception of two lesional biopsy specimens from patients with urticaria, all biopsy specimens were taken from the

arm. Elliptical skin biopsies were performed after infiltration around but not into the biopsy site with local anaesthetic (1% lidocaine without epinephrine), fixed in Carnoy's fixative (methanol-chloroform-acetic acid, 60:30:10, vol/vol) overnight, and processed in paraffin.

## **Immunohistochemistry**

Tissue sections (4  $\mu\text{m}$ ) were mounted on glass slides and dried. Sections were dewaxed in xylene over three 5-minute periods and rehydrated with graded ethanol (100%, 95%, 80%, 70%, 50%) and water, each for a 3-minute period. Endogenous peroxidase was inhibited by incubating the sections in methanol containing 0.6% hydrogen peroxide for 30 minutes. Nonspecific staining was reduced by incubating sections with 10% heat-inactivated normal goat serum for 1 hour at 22° C. Sections were washed with Tris-buffered saline, pH 7.4, for 5 minutes and then incubated with biotinylated monoclonal murine anti-chymase antibody (B7, 4  $\mu\text{g}/\text{ml}$ ) at 4° C overnight. Sections were washed with Tris-buffered saline and peroxidase-conjugated streptavidin (1:50, vol/vol dilution), applied for 1 hour at room temperature.<sup>11</sup> The peroxidase reaction was visualized with freshly made 3-amino-9-ethylcarbazole (0.2 mg/ml in 0.1 mol/L, pH 5.2, acetate buffer) containing 0.01% hydrogen peroxide applied for 5 minutes. Chymase-positive mast cells stained a reddish brown color. Sections were washed, and alkaline phosphatase-conjugated murine monoclonal anti-tryptase antibody (G3AP, 1.5  $\mu\text{g}/\text{ml}$ ) was then applied overnight at 4° C. Immunostaining was visualized with fast blue RR chromogen made up as follows: 0.2 mol/L Tris-HCl, pH 8.2 (9.8 ml), was added to naphthol ASMX phosphate (2 mg in 0.2 ml dimethylformamide), followed by fast blue RR salt (10 mg). The solution was immediately filtered and applied to each section for 15 minutes. A blue color alone developed in mast cells containing tryptase MC<sub>T</sub> cells only; these were previously unstained by the anti-chymase antibody. Sections were then thoroughly washed with Tris-buffered saline pH 7.4, and mounted in aqueous medium containing 90% glycerol in phosphate-buffered saline, pH 7.4. Controls included omission of primary antibody and use of an IgG monoclonal antibody (MOPC315) of irrelevant specificity. In addition, double-labeling immunohistochemistry of sections from normal human lung, tonsil, and intestine was performed concurrently with skin samples as controls for mast cells containing tryptase and chymase (MC<sub>TC</sub>) and MC<sub>T</sub> cells.

## **Quantification**

Sections were coded and read in blinded fashion by one investigator. Mast cells were counted under light microscopy at  $\times 160$  magnification. Mast cell concentrations were obtained with using a Zeiss Videoplan apparatus (Carl Zeiss Inc., Thornwood, N.Y.) and calculated according to the following formula:  $\text{mast cells}/\text{mm}^3 = [\text{mast cells}/\text{mm}^2] \cdot [9.5 \mu\text{m} + 4 \mu\text{m}]$ , where 9.5  $\mu\text{m}$  represents the mean mast cell diameter and 4  $\mu\text{m}$  represents the tissue section thickness. Mast cell numbers were quantified in two areas of dermis (1) 0 to 183  $\mu\text{m}$  and (2) 183 to 366  $\mu\text{m}$  from the dermo-epidermal junction. Percentages of MC<sub>TC</sub> and MC<sub>T</sub> cells were also calculated.

## **Statistics**

Differences in mast cell number among lesional, nonlesional, and control skin for each area counted (i.e., 0 to 183  $\mu\text{m}$  and 183 to 366  $\mu\text{m}$ ) were calculated by using Student's unpaired *t* test.

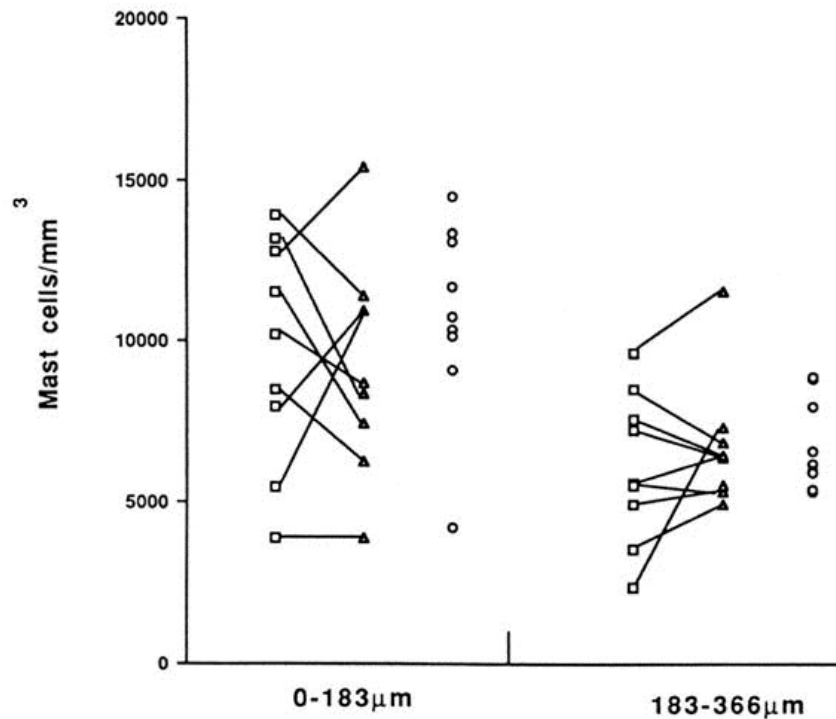
## RESULTS

No significant differences were observed among lesional, nonlesional, or control skin at either 0 to 183  $\mu\text{m}$  or 183 to 366  $\mu\text{m}$  from the dermo-epidermal junction (Table I, Fig. 1). With the exception of samples from two patients with urticaria (in which 95% and 96% of mast cells, respectively, were of the MC<sub>TC</sub> type in nonlesional skin), in other samples (from both patients with urticaria and control subjects) more than 99% of mast cells present were of the MC<sub>TC</sub> type.

**TABLE I.** Mast cell numbers in lesional, nonlesional, and control skin

| Depth from dermo-epidermal junction | Lesional ( <i>n</i> = 11) | Nonlesional ( <i>n</i> = 9) | Control ( <i>n</i> = 10) |
|-------------------------------------|---------------------------|-----------------------------|--------------------------|
| Depth = 0 to 186 $\mu\text{m}$      |                           |                             |                          |
| Range                               | 3,888-13,690              | 3,901-15,358                | 4,234-14,483             |
| Median                              | 8,489                     | 8,693                       | 10,584                   |
| Mean ( $\pm$ SEM)                   | 9,299 $\pm$ 1,009         | 9,018 $\pm$ 1,096           | 10,735 $\pm$ 900         |
| Depth = 186 to 366 $\mu\text{m}$    |                           |                             |                          |
| Range                               | 2,345-9,640               | 4,941-11,567                | 5,377-8,920              |
| Median                              | 5,599                     | 6,426                       | 6,377                    |
| Mean $\pm$ SEM                      | 6,044 $\pm$ 661           | 6,597 $\pm$ 682             | 6,782 $\pm$ 420          |

Figures denote mast cell numbers per cubic millimeter. No significant differences were observed among any of the groups examined (Student's *t* test, *p* > 0.1)



**FIGURE 1.** Scatter plot showing paired data points from lesional (□) and nonlesional (△) skin, together with data points from skin of healthy control subjects (○) 0-183  $\mu\text{m}$  and 183 to 366  $\mu\text{m}$  from the dermo-epidermal junction. No significant differences were observed between these groups (Student's unpaired *t* test).

## DISCUSSION

Chymase and tryptase are located almost exclusively in mast cells, basophils containing negligible amounts of tryptase (0.04 pg/cell) and undetectable amounts of chymase. The alkaline phosphatase-conjugated G3 antibody stains only mast cells.<sup>11</sup> Similarly, eosinophils, neutrophils, monocytes, and lymphocytes have no detectable tryptase.<sup>12, 13</sup> Thus immunohistochemical localization of these two enzymes provides a discriminating marker for human mast cells. Although chymase and tryptase are released during degranulation of allergen-stimulated mast cells, mast cells remain immunohistochemically detectable with this method because they are not completely depleted of their protease content and perhaps because a portion of the released proteases remain closely associated with the cells (Schwartz LB, Irani AA. Unpublished data).

The data indicate that the numbers of mast cells in nonlesional and control skin are similar and are in agreement with the findings of a previous study in which mast cell numbers were quantified with toluidine blue.<sup>14</sup> The finding that numbers of mast cells in lesional skin are comparable to those in control and nonlesional skin contrasts with the previously reported finding that a 10-fold increase in mast cells was observed in lesional urticarial skin.<sup>15</sup> This latter study, however, used Leder's chloracetate esterase reaction for mast cell detection,<sup>16</sup> which stains neutrophils in addition to mast cells. Hence, in the presence of neutrophilic infiltrates, which are well-documented in urticarial lesions,<sup>15, 17, 18, 19</sup> mast cell numbers may be overestimated,<sup>20</sup> thus accounting for the discrepancy.

Observed increases in nonlesional and lesional skin histamine content cannot therefore, on the basis of current data, be explained by an increase in the number of mast cells. The increase in lesional skin histamine may be due to recruitment of basophils into skin, as has been demonstrated in the late-phase cutaneous reaction to antigen with the skin chamber model.<sup>21</sup> Others,<sup>15</sup> however, found no histologic evidence of basophils in lesional chronic urticaria, although it is possible that once recruited to extravascular sites, basophils degranulate too rapidly to be distinguished morphologically. To date, no basophil-specific epitopes have been identified, which would allow more definitive quantification of tissue basophils in urticaria. Alternatively, production of histamine by mast cells may be generally increased in urticaria because of repeated mast cell degranulation and histamine release, possibly resulting in induction of histidine decarboxylase and greater histamine content per mast cell. This phenomenon might also account for the putative increases in nonlesional skin histamine, when amounts released could be subclinical.

Normal skin has been previously reported to contain MC<sub>TC</sub> cells exclusively, and thus the current finding that more than 99% of skin mast cells are MC<sub>TC</sub> cells in healthy control subjects is expected. In systemic mastocytosis MC<sub>TC</sub> cells again are the predominant type of mast cells present.<sup>22</sup> The majority of cutaneous mast cells in patients with chronic idiopathic urticaria similarly demonstrated the MC<sub>TC</sub> phenotype. This observation may have functional significance in urticaria, given the susceptibility of skin MC<sub>TC</sub> cells to activation by neuropeptides such as substance P.<sup>23, 24, 25, 26</sup>

In conclusion, when a specific, sensitive double-labeling immunohistochemical technique is used, mast cell numbers in nonlesional and lesional skin from patients with chronic idiopathic urticaria are similar to those of control subjects. These data suggest that previously documented increases in skin histamine in patients with urticaria are unlikely to be due to a change in

numbers of skin mast cells and may alternatively reflect an increased histamine content per mast cell, an increased activation state of the mast cells, or recruitment of basophils into the skin.

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